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(54) POLYMER COMPOSITION FOR FORMING SURFACE OF BIOSENSOR

(57) A polymer composition is provided, which contains a polymer or copolymer having a mercapto group at one end and functional group or ligand at the other end and also having a polyethylene glycol segment.

Such a composition can form a biosensor surface of reduced non-specific adsorbability for proteins and the like.

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Description**Technical Field**

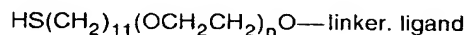
[0001] This invention relates to a polymer composition for forming surface of a biosensor utilizing surface plasmon resonance (SPR), biosensor chip surfaces onto which such a polymer is absorbed (or adsorbed), and a method for producing biosensor chips using the composition.

Background Art

[0002] Surface plasmon resonance (SPR) is sensitive to changes in the index of refraction at and near the surface of a metal film (e.g., see A. Szabo, et al., *Curr. Opin. Strct. Biol.* 5(1995)699—705). SPR enables an in situ observation of processes occurring between a surface and complex biological solutions that allows, e.g., the acquisition of data in real time without requiring tagging of the analytes. That is a technique convenient for obtaining both kinetic and thermodynamic parameters. Where biomolecules immobilized at a surface lead to changes in the index of refraction of the layer on which they are immobilized, SPR can also detect conformational changes of said biomolecules.

[0003] As a typical biosensor chip having this kind of surface, BIACORE which is commercially available from Amersham Pharmacia Biotech., Inc. can be named, which is provided in form of chips in which a matrix of dextran with carboxylated ends is immobilized on a translucent gold film. Such a dextran matrix exhibits a certain extent of resistance to non-specific adsorption of proteins, but because it has a considerable thickness (ca. 100nm), in certain occasions it may have adverse influence on the thermodynamic and kinetic parameters where, for example, partitioning of the analyte in the matrix takes place.

[0004] On the other hand, E. Ostuni, et al., *Colloids and Surface B: Biointerfaces* 15(1999)3—30 discloses chips comprising a polymer represented by a general formula:



(wherein n is an integer of 2 — 7)

as carried in the form of a self-assembled monolayer on a gold layer (12 nm) which is applied on a titanium thin layer (1 — 5 nm) vacuum-evaporated on a glass support. In said review, the authors suggest that the monolayer formed of said polymer having 2 — 7 ethylene glycol units exhibits resistance to adsorption of proteins. They further suggest that the above result would disagree with the theoretical prediction: for example, when polyethylene glycol (PEG) is grafted on a trichlorovinylsilane-modified glass, the shorter becomes the PEG chain, the more its resistance to adsorption of proteins would drop (S. I. Jeon, et al., *J. Colloid Interface Sci.* 142(1991)149—158).

[0005] However, polymers of small PEG units proposed by E. Ostuni, et al. in certain cases non-specifically adsorb specific proteins (e.g., those having positive electric charge as a whole), under the influence of metallic film surface.

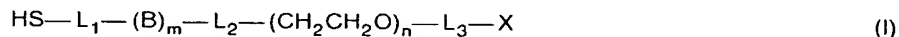
Disclosure of the Invention

[0006] The object of the present invention is to provide a polymer composition utilizing SPR, which can significantly reduce non-specific adsorption of any proteins irrelevantly to their kinds, and still can remove or significantly improve the defect inherent in the use of dextran matrix in BIACORE.

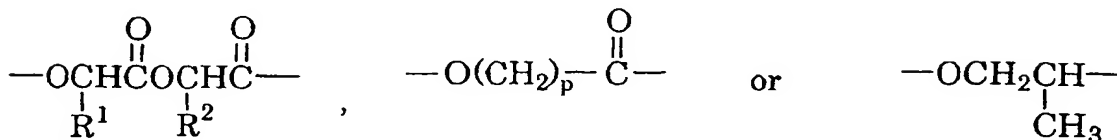
[0007] We have discovered that non-specific adsorption of biocomponents including proteins could be significantly reduced when a specific polymer having PEO segments which are regarded to be classified as a polymer, rather than an oligomer, is chemically absorbed (or adsorbed) onto a metal film, although so formed polymer matrix layer would be expected to have a far greater thickness than that of the matrix of said E. Ostuni, et al.

[0008] Accordingly, the present invention provides a polymer composition for forming surface of a biosensor utilizing surface plasmon resonance (SPR), which comprises the specific polymer (i.e., a polymer represented by the following general formula (I) as an active ingredient; and biosensor chips for detecting the SPR, which have adsorbed said polymer onto their surfaces:

general formula (I):



in which L_1 , L_2 and L_3 each represents a valence bond or linker independently of each other, but where m is zero (0), L_1 and L_2 may together form a valence bond or a linker;
 B stands for a formula,



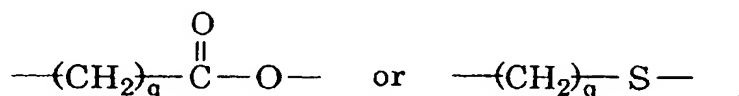
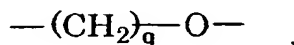
where R^1 and R^2 each independently stands for hydrogen atom or C_{1-5} alkyl group and p is an integer of 2 — 5;
 x stands for a hydrogen atom, functional group or ligand;
 m is an integer of 0 — 10,000, and
 n is an integer of 10 — 20,000.

[0009] As another embodiment, the present invention provides a method for production of biosensor chips utilizing SPR, which is characterized by treating sensor chips having a metal surface selected from the group consisting of gold, silver, platinum and aluminum, with a solution or suspension of the polymer composition containing a polymer represented by above general formula (I), causing said surface to chemically adsorb or chemically absorb the polymer via the mercapto group in said polymer, and if necessary covalently bonding a ligand thereto via the functional group X in the polymer.

[0010] Also as still another embodiment, the present invention provides use of polymers represented by the general formula (I) for forming surfaces of biosensor chips utilizing SPR.

Best Mode for Carrying Out the Invention

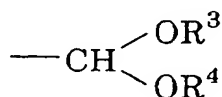
[0011] Most of the polymers represented by the general formula (I) are known per se. Also those covered by the same formula which are deemed to be novel can be prepared in the manner similar to that for preparing the known polymers. Of these polymers, the preferred ones are those whose m in the general formula (I) ranges 0 — 10,000; L_1 stands for a valence bond or a linker of a formula,



or where m is zero (0), L_1 and L_2 may together be a linker as defined above as to L_1 , wherein q being an integer of 2 — 6; and where m is other than zero (0), L_2 is $-\text{O}-$ or $-\text{O}-\text{CH}_2\text{CH}_2-\text{O}-$;

L_3 is a valence bond or $-(\text{CH}_2)_r-$, r being an integer of 1 — 6; and

x stands for a hydrogen atom, a functional group selected from the group consisting of aldehyde ($-\text{CHO}$), hydroxyl, amino, groups represented by the following formula

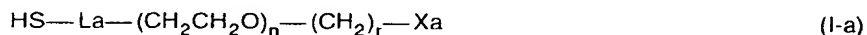


(in which R^3 and R^4 stand for either C_{1-10} alkyl group independently of each other or are atomic groups together forming an optionally C_{1-6} alkyl-substituted ethylene group), groups of the formula $-\text{NR}^5\text{R}^6$ (in which R^5 and R^6 stand

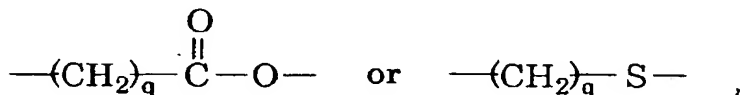
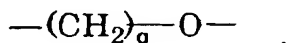
for a hydrogen atom or a C₁₋₆ alkyl group independently of each other, provided either one of R⁵ and R⁶ is not hydrogen), acryloyl, methacryloyl, vinylbenzyl, allyl and p-toluenesulfonyl; or a ligand selected from the group consisting of sugar residue, biotin, antigen or antibody and nucleic acid.

[0012] Of these preferred polymers which are used in the present invention, those represented by the following general formula (I-a) can be named as examples of the polymers whose m is zero (0) referring to the general formula (I).

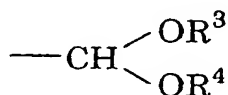
General formula (I-a):



in which La stands for



q being an integer of 2 — 6,
Xa is aldehyde group or



(where R³ and R⁴ either stand for C₁₋₁₀ alkyl independently of each other or are atomic groups together forming an optionally C₁₋₆ alkyl-substituted ethylene group),

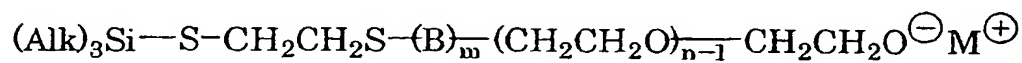
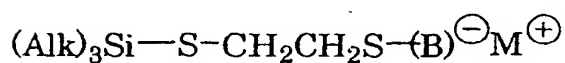
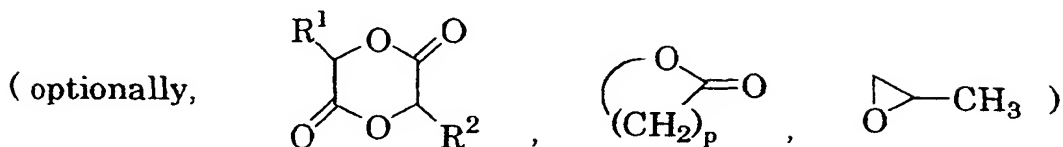
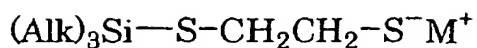
n is an integer of 10 — 20,000, and

r is an integer of 1 — 6.

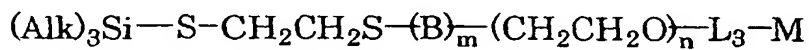
[0013] These polymers can be prepared by the methods disclosed in, for example, WO 96/32434, WO 96/33233, WO 97/06202, JP 11(1999)-322916 A and JP 11(1999)-322917 A, or if necessary modifying so prepared polymers or their precursors by methods known per se.

[0014] Although not in limitative sense, it is convenient to perform preparation of a polymer of the general formula (I) having a mercapto group at one end and a functional group or ligand at the other end, following the reaction schemes given hereafter, in which the individual steps can be practiced according to the methods known per se.

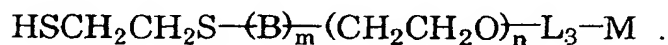
Scheme I (see: JP 11(1999)-322917 A):



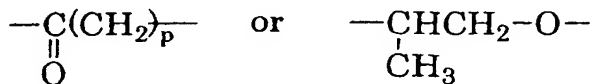
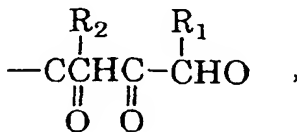
electrophilic agent A-X



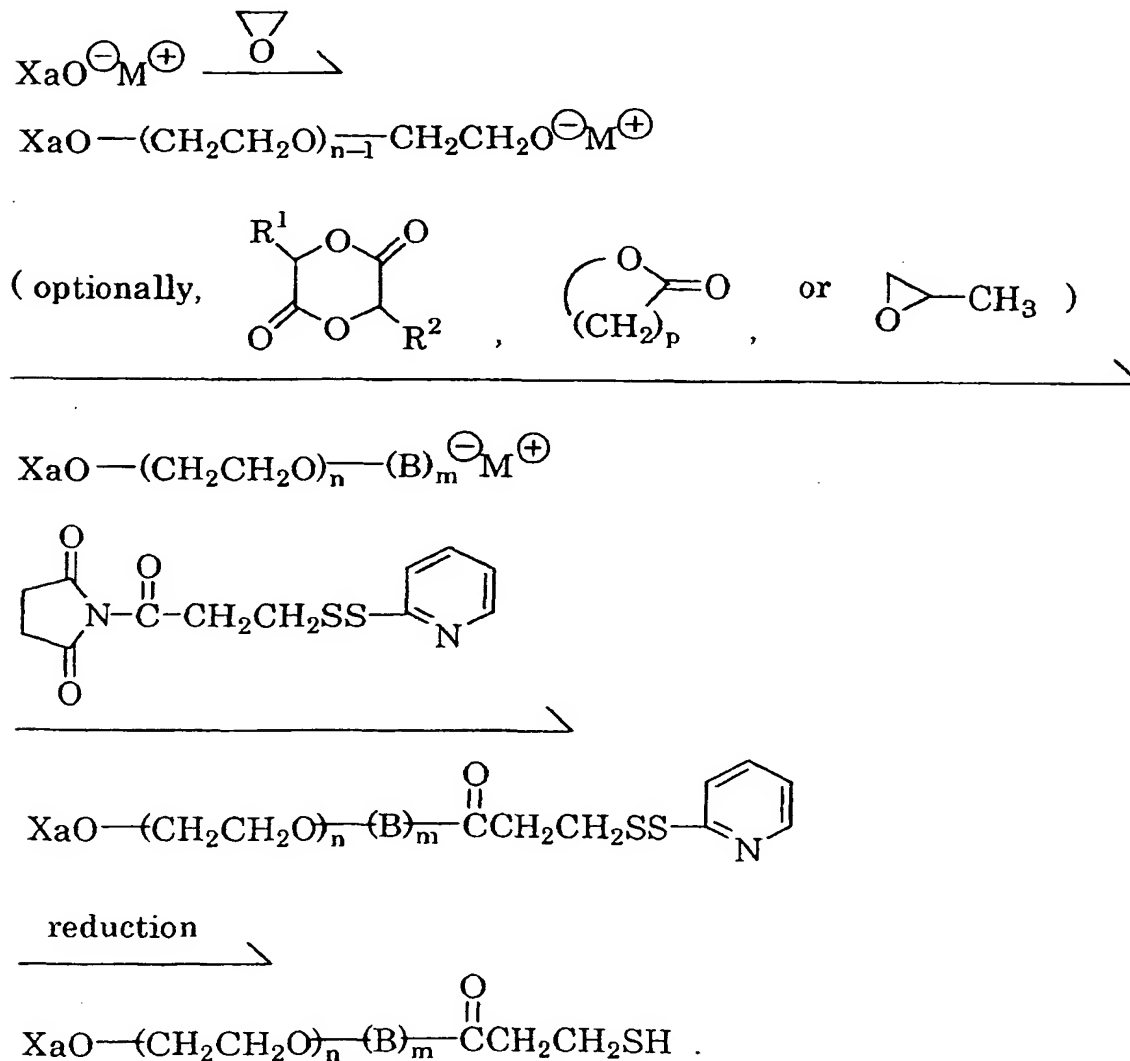
desilylation



[0015] In the above formulae, Alk signifies C₁₋₆ alkyl M⁺ is a potassium ion; A is a leaving group such as halogen; R¹, R², X, n, m and L₃ have the above-defined significations; and B is



Scheme II (concerning preparation of the precursor polymers, see WO96/32434, WO96/33233 and WO97/06202):



In the above formulae, Xa is X-L₃, and all other abbreviations and signs have the same significations as earlier given.

[0016] In the present specification, alkyl groups mean optionally branched alkyl groups, and C₁₋₆ or C₁₋₁₀ put before the words, "alkyl groups" mean that the number of carbon atoms in the alkyl groups are 1-6 or 1-10, respectively. Therefore, although not in limitative sense, as examples of such alkyl groups, methyl, ethyl, n-propyl, i-propyl, n-butyl, sec-butyl, tert-butyl, n-pentyl, n-hexyl, n-octyl, n-decyl and the like can be named.

[0017] A ligand as referred to in this invention can be the other member of a bonding pair capable of constituting a biological conjugate. For instance, biotin in biotin-avidin conjugate, sugar in sugar-lectin conjugate, antigen or antibody in antigen-antibody conjugate, and either of the nucleic acid in nucleic acid-nucleic acid hybrid may be named. These ligands can be introduced into one end of the polymer of the general formula (I), utilizing the reaction between the functional group (e.g., aldehyde group) present at said one end of the polymer and amino group in the ligand. It is also permissible to conduct such introduction of ligands after immobilizing a polymer having a functional group at one end on a metal surface as described later.

[0018] According to the invention, the number "n" of the polyethylene segment is at least 10, preferably 10 — 2500,

inter alia, 50 — 500.

[0019] As above, the polymers of the general formula (I) each having a mercapto group at one end are stably soluble or dispersible in various solvents, for example, tetrahydrofuran, lower alkanol (e.g., ethanol), dimethylformamide, toluene, xylene, etc. Solutions or dispersions of polymers of the general formula (I) in these solvents constitute one embodiment of polymer compositions of the present invention for forming surfaces of biosensors utilizing SPR. Such a polymer composition may contain more than one kind of polymer, and where necessary, other additive(s), for example, an inorganic salt such as potassium sulfate may be incorporated, to enable immobilization of the polymer or polymers on the surface at a high density.

[0020] Thus formulated polymer compositions in general may take any form, which are contacted with chips formed of glass or silicon wafer on which a layer of gold, silver, platinum, palladium, aluminum or the like is deposited, where necessary via a titanium film which is vacuum-evaporated on the wafer to improve adhesivity of gold, silver, aluminum, etc., and whereby chemically absorbed (or adsorbed) onto the gold, silver or platinum. Chemical absorption (or adsorption) is not restricted by theories, but is understood to occur through formation of metal-thiolate (which may hereafter be referred to as conjugate) through reaction of mercapto groups of the polymers with the metal surface. This absorption (or adsorption) is conducted, for example, by immersing a support carrying a translucent gold film in aforesaid polymer solution or flowing the polymer solution over the support surface. While the optimum treating temperature varies depending on the kind of the polymer used, generally it can be within the range between melting point and boiling point of the solvent used. Considering operational ease, temperatures in the vicinity of room temperature are convenient. When treated at around the room temperature, normally sufficient contact time between the metal surface and polymer solution is around 30 minutes, while it may be prolonged to 1 — 24 hours or even more. The polymer or polymers of the general formula (I) are thus absorbed (or adsorbed) onto the metal surface. If required, unabsorbed (or unadsorbed) polymer may be washed away.

[0021] The polymer concentration in such a polymer solution is not critical so long as the polymer is completely dissolved or uniformly dispersed, while generally preferred range is 10 — 200 µg/ml.

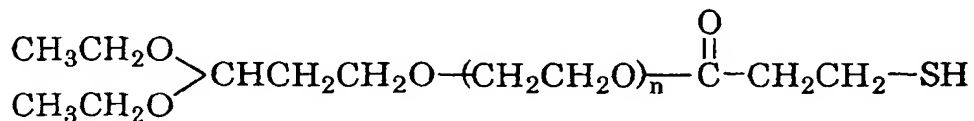
[0022] The metal surface (e.g., surface of a sensor chip) onto which a polymer or polymers of the general formula (I) are chemically absorbed (or adsorbed) via the mercapto group(s) as provided through above procedures is covered with polyethylene glycol segment(s) of adequate chain length(s), and is substantially free of direct influences from the metal surface and the surface charge is expected to become zero or endlessly approach zero. Consequently, at the surface formed of the composition of the invention on the metal, non-specific adsorption of biomolecular species (e.g., positively charged protein) attributable to surface electric charge does not substantially take place, while such non-specific adsorption is occasionally observed at metal surfaces covered with alkylene thiol having a terminal composed of, for example, less than 10 ethylene glycol units.

[0023] The present invention is more specifically explained hereinafter, referring to concrete examples which, however, are not intended to limit the invention thereto. In the following, furthermore, polyethylene segment is abbreviated as PEG.

Production Example 1

[0024] Synthesis of a polymer having a mercapto group at one end and an acetal group-containing heterotelechelic PEG at the other end

[0025] 1. Synthesis of acetal-PEG-SH (Mn=5000)



[0026] Distilled tetrahydrofuran (THF) 20 ml, and 3,3-diethoxy-1-propanol, an initiator, 0.2 mmol (0.032 ml) were added to an argon-substituted reactor, and further an equivalent amount of potassium naphthalene was added, followed by 15 minutes' stirring to conduct metallization. Then ethylene oxide 22.7 mmol (1.135 ml) was added, followed by two days' stirring to conduct polymerization. As a reaction-suspending agent, N-succinimido-3-(2-pyridylthio)propionate (SPDP) 0.4 mmol (0.125 g) was dissolved in a small amount of distilled THF and into the resultant solution said polymerization reaction solution was dropped under cooling with ice, through an isopiestic dropping funnel. After an overnight stirring the reaction was suspended and the polymer was recovered by the series of operations as washing with saturated saline solution, extraction with chloroform, reprecipitation from ethylene and freeze drying from benzene. The construction of the recovered polymer was confirmed with ¹H-NMR,

and the amount of SPDP residue introduced to the polymer terminal was confirmed by UV absorption of 2-thiopyridone which was released upon reaction with 2-mercaptoethanol.

[0027] PEG-SS-Py 2.0×10^{-2} mmol (100 mg) was dissolved in distilled water 4 ml, to which further 5 molar times thereof of dithiothreitol 0.1 mmol (15.42 mg) was added, followed by 30 minutes' stirring at room temperature. After the reaction, the polymer (hereafter abbreviated as PEG 5000) was recovered through a series of operations as washing with saturated saline water, extraction with chloroform and reprecipitation from ether. The construction of the recovered polymer was confirmed with $^1\text{H-NMR}$ and the amount of terminal SH group was determined by the reaction with 2-pyridyldisulfide (2-PDS).

[0028] Furthermore, substantially the same operations as above were repeated except that the feed amount of ethylene oxide was decreased and increased in respective runs, to produce polymers of $M_n=2,000$ and $M_n=10,000$, respectively, which are hereafter abbreviated as PEG 2,000 and PEG 10,000. M_n represents the molecular weight of respective PEG segments.

Example 1: Immobilization of PEG onto JI sensor chips

[0029] Each of the polymers obtained in above Production Example 1 was dissolved in a boron buffer of pH=8.0 and prepared solutions of 1, 5, 10, 20 and 50 $\mu\text{g/ml}$ as converted to SH-containing PEG (the concentration levels given herein are those regarding SH-containing PEG, which are approximately twice in terms of PEG concentration levels because the actual samples contain OH end groups). Flowing those solutions over JI sensor chip (BIAcore-originated) at a flow rate of 5 $\mu\text{l/min}$. for an hour, further 0.5% sodium dodecylsulfate (SDS) was flown at a rate of 10 $\mu\text{l/min}$. for 30 minutes to wash non-specific adsorbate away, and the substance bound onto the gold surfaces of the chips was quantified.

[0030] The results of the quantification are shown as RU saturation values, which were obtained as follows. RU values [a coefficient so set that 1 ng/mm^2 of the substance adsorbed onto said surface corresponds to 1000RU by, in said BIAcore biosensor system utilizing surface plasmon resonance, irradiating laser beam having a specific wavelength in such a manner to cause its total internal reflection and measuring the index of refraction) before adsorption of PEG 2000, PEG 5000 and PEG 10000, respectively (RU start) and after the adsorption and washing with SDS (RU_{SDS}) were determined, and the values obtained by subtracting respective RU start values from RU_{SDS} values were indicated as RU saturation values. Further, these RU saturation values were divided by the molecular weight of used polymer and the number of adsorbed polymer chains per 1 nm^2 was indicated as the immobilized polymer density.

[0031] The results are collectively shown in the following Table 1.

Table 1:

Characteristics of Each of Treated Surfaces			
Polymer	Polymer Concentration Flown Over Surface ($\mu\text{g/ml}$)	RU Saturation Value	Immobilized Polymer Density (number of chains/ nm^2)
PEG2000	100	1500	0.56
PEG5000	50	1500	0.18
PEG10000	100	2300	0.14

Example 2: Adsorption characteristics of various proteins onto adsorptive polymer surfaces

[0032] Onto the surfaces of those JI sensor chips having the treated surfaces as prepared in Example 1 and onto those of CM5 (prepared by adsorbing dextran onto JI sensor chips: BIAcore-originated), each 1 mg/ml (10 mM of PBS, pH 7.4) solution of bovine serum albumin (BSA: molecular weight 69000; isoelectric point 4.9), lysozyme (Lysozyme: molecular weight 14300; isoelectric point 11) and avidin (Avidin: molecular weight 68000; isoelectric point 10 — 10.5) was flown at a rate of 5 $\mu\text{l/ml}$. for 20 minutes, to draw an adsorption curve each. Thereafter the procedures as described in Example 1 was followed to provide each of RU values. The adsorption amounts (RU) of BSA, Lysozyme and Avidin were as shown in the following Tables 2, 3 and 4, respectively.

Table 2:

BSA Adsorption [RU] on Each Surface	
Polymer	Immediately after protein adsorption
PEG2000	1154.7

Table 2: (continued)

BSA Adsorption [RU] on Each Surface	
Polymer	Immediately after protein adsorption
PEG5000	499.6
PEG10000	645.6
CM5	123.5

Table 3:

Lysozyme Adsorption [RU] on Each Surface	
Polymer	Immediately after protein adsorption
PEG2000	1358.0
PEG5000	1038.0
PEG10000	1413.4
CM5	18775.0

Table 4:

Avidin Adsorption [RU] on Each Surface	
Polymer	Immediately after protein adsorption
PEG2000	2364.2
PEG5000	1710.4
PEG10000	1784.4
CM5	32694.9

[0033] Those results indicated in above tables show significantly reduced non-specific adsorption of various proteins onto PEG 2000, PEG 5000 and PEG 10000, from that onto CM5 with dextran-adsorbed surface. In particular, in respect of Lysozyme and Avidin, the adsorption values decreased to about 1/15 — 1/20.

Industrial Utilizability

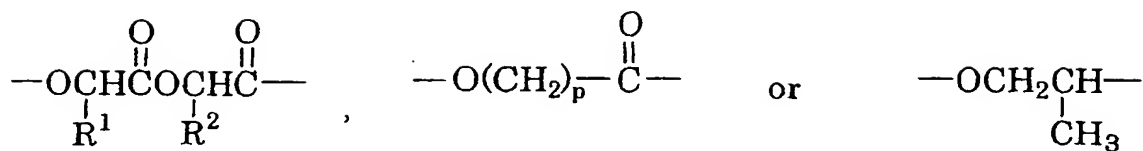
[0034] According to the present invention, sensor chips in which non-specific adsorption of biocomponents onto the biosensor surfaces are eliminated are provided. They, therefore, can be used by biosensor producing industries and for diagnoses using such sensors.

Claims

1. A polymer composition for forming surface of a biosensor utilizing surface plasmon resonance (SPR), which comprises a polymer represented by the following general formula (I) as an active ingredient, solvent and optionally an adjuvant or adjuvants:

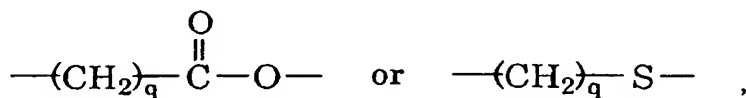
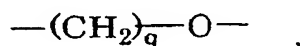


in which L_1 , L_2 and L_3 each represents a valence bond or linker independently of each other, but where m is zero (0), L_1 and L_2 may together form a valence bond or a linker;
B stands for a formula,



where R¹ and R² each independently stands for hydrogen atom or C₁₋₅ alkyl group and p is an integer of 2 - 5;
 x stands for a hydrogen atom, functional group or ligand;
 m is an integer of 0 - 10,000, and
 n is an integer of 10- 20,000.

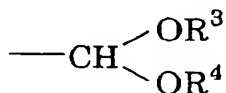
2. A polymer composition according to Claim 1, in which the surface of said sensor is made of at least one metal selected from the group consisting of gold, silver, platinum and aluminum, and the polymer is chemically absorbed into (or adsorbed onto) said surface via the mercapto group in the polymer molecular chain.
3. A polymer composition according to Claim 1 or 2, in which, referring to the formula (I),
 m is 0 - 10,000,
 L₁ stands for a valence bond or a linker of a formula,



or where m is zero (0), L₁ and L₂ may together be a linker as defined above as to L₁, wherein q being an integer of 2 - 6; and where m is other than zero (0), L₂ is -O- or -O-CH₂CH₂-O;

L₃ is a valence bond or -(CH₂)_r-, r being an integer of 1 - 6; and

X stands for a hydrogen atom, a functional group selected from the group consisting of aldehyde (-CHO), hydroxyl, amino, groups represented by the following formula

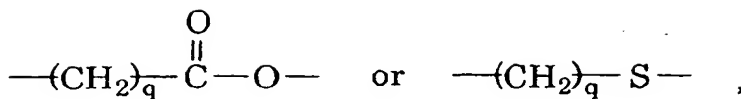
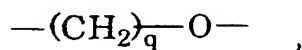


(in which R³ and R⁴ stand for either C₁₋₁₀ alkyl group independently of each other or are atomic groups together forming an optionally C₁₋₆ alkyl-substituted ethylene group), groups of the formula -NR⁵R⁶ (in which R⁵ and R⁶ stand for a hydrogen atom or a C₁₋₆ alkyl group independently of each other, provided either one of R⁵ and R⁶ is not hydrogen), acryloyl, methacryloyl, vinylbenzyl, allyl and p-toluenesulfonyl; or a ligand selected from the group consisting of sugar residue, biotin, antigen or antibody and nucleic acid.

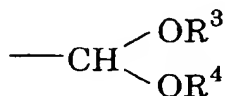
4. A polymer composition according to any one of Claim 1 - 3, in which the polymers represented by the formula (I) are those expressed by the following general formula (I-a)



in which La stands for



q being an integer of 2 - 6,
Xa is aldehyde group or



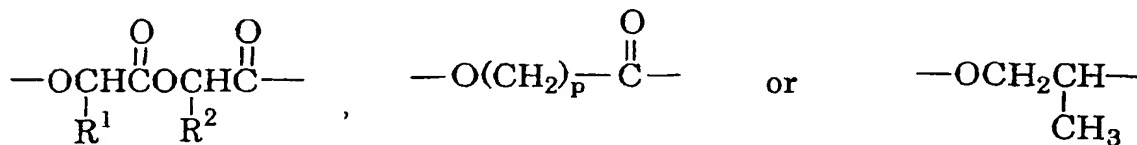
(where R³ and R⁴ either stand for C₁₋₁₀ alkyl independently of each other or are atomic groups together forming an optionally C₁₋₆ alkyl-substituted ethylene group),
n is an integer of 10 - 20,000, and
r is an integer of 1 - 6.

5. Biosensor chips for detecting surface plasmon resonance (SPR), in which a polymer represented by the following general formula (I) is absorbed (or adsorbed) onto the sensor chip surfaces via the mercapto group present at one end of said polymer:

general formula (I)



in which L₁, L₂ and L₃ represent valence bonds or linkers independently of each other, but where m is zero (0), L₁ and L₂ may together form a valence bond or a linker;
B stands for a formula,



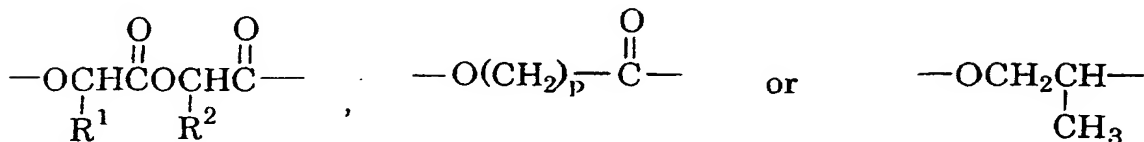
where R¹ and R² each independently stands for hydrogen atom or C₁₋₅ alkyl group and p is an integer of 2 - 5;
x stands for a hydrogen atom, functional group or ligand;
m is an integer of 0 - 10,000, and
n is an integer of 10 - 20,000.

6. Biosensor chips according to Claim 5, in which surfaces of the sensor chips are formed of at least one metal selected from the group consisting of gold, silver, platinum and aluminum.
7. Use of a polymer represented by the following general formula (I), for forming surfaces of biosensor chips utilizing surface plasmon resonance (SPR):

general formula (I)



in which L_1 , L_2 and L_3 represent valence bonds or linkers independently of each other, but where m is zero (0), L_1 and L_2 may together form a valence bond or a linker;
 B stands for a formula,

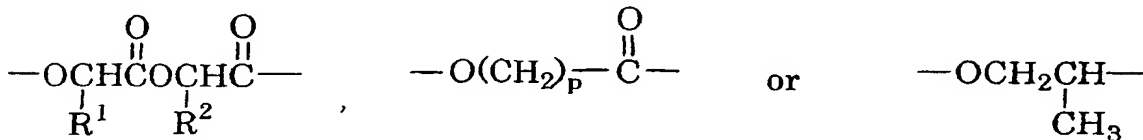


where R^1 and R^2 each independently stands for hydrogen atom or C_{1-5} alkyl group and p is an integer of 2 - 5;
 x stands for a hydrogen atom, functional group or ligand;
 m is an integer of 0 - 10,000, and
 n is an integer of 10 - 20,000.

8. A method for making biosensor chips utilizing surface plasmon resonance (SPR), which comprises contacting a solution or dispersion of a polymer represented by the following general formula (I):



in which L_1 , L_2 and L_3 represent valence bonds or linkers independently of each other, but where m is zero (0), L_1 and L_2 may together form a valence bond or a linker;
 B stands for a formula,



where R^1 and R^2 each independently stands for hydrogen atom or C_{1-5} alkyl group and p is an integer of 2 - 5;
 x stands for a hydrogen atom, functional group or ligand;
 m is an integer of 0 - 10,000, and
 n is an integer of 10 - 20,000

with surfaces of sensor chips, said surfaces being formed of at least one metal selected from the group consisting of gold, silver, platinum and aluminum, whereby causing the polymer to be chemically adsorbed onto or chemically absorbed into said surfaces via the mercapto group present at one end of said polymer, and if necessary covalently bonding a ligand thereto via the functional group X .

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP01/03886

A. CLASSIFICATION OF SUBJECT MATTER Int.Cl. ⁷ G01N33/543, G01N21/76		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) Int.Cl. ⁷ G01N33/543, G01N21/76		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Jitsuyo Shinan Koho 1922-1996 Toroku Jitsuyo Shinan Koho 1994-2001 Kokai Jitsuyo Shinan Koho 1971-2001 Jitsuyo Shinan Toroku Koho 1996-2001		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CA (STN)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	OSTUNI, E. et al., "The interaction of proteins and cells with self-assembled monolayers of alkanethiolates on gold and silver", Colloids and Surfaces B: Biointerfaces Vol.15 (1999) pp.3-30	1-8
Y	PAVER, K. D. et al., "SPR analysis of the total reduction of protein adsorption to surfaces coated with mixtures of long- and short-chain polyethylene oxide block copolymers" Biomaterials Vol.20, No.9 (1999) pp.885-890 Abstract	1-8
Y	WO 96/32434 A (KATAOKA K), 17 October, 1996 (17.10.96), & JP 8-530892 A & EP 852243 A & US 5973069 A	1-8
Y	WO 96/33233 A (KATAOKA K), 24 October, 1996 (24.10.96), & JP 8-531619 A & EP 822217 A & US 5925720 A	1-8
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 09 July, 2001 (09.07.01)		Date of mailing of the international search report 24 July, 2001 (24.07.01)
Name and mailing address of the ISA/ Japanese Patent Office		Authorized officer
Facsimile No.		Telephone No.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP01/03886

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97/06202 A (KATAOKA K), 20 February, 1997 (20.02.97), & JP 9-508315 A & EP 844269 A & US 5929177 A	1-8

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